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Farnesol and Cyclic AMP Signaling Effects on the Hypha-to-Yeast Transition in *Candida albicans*

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***Candida albicans*, a fungal pathogen of humans, regulates its morphology in response to many environmental cues and this morphological plasticity contributes to virulence. Farnesol, an autoregulatory molecule produced by *C. albicans*, inhibits the induction of hyphal growth by inhibiting adenylate cyclase (Cyr1). The role of farnesol and Cyr1 in controlling the maintenance of hyphal growth has been less clear. Here, we demonstrate that preformed hyphae transition to growth as yeast in response to farnesol and that strains with increased cyclic AMP (cAMP) signaling exhibit more resistance to farnesol. Exogenous farnesol did not induce the hypha-to-yeast transition in mutants lacking the Tup1 or Nrg1 transcriptional repressors in embedded conditions. Although body temperature is not required for embedded hyphal growth, we found that the effect of farnesol on the hypha-to-yeast transition varies inversely with temperature. Our model of Cyr1 activity being required for filamentation is also supported by our liquid assay data, which show increased yeast formation when preformed filaments are treated with farnesol. Together, these data suggest that farnesol can modulate morphology in preformed hyphal cells and that the repression of hyphal growth maintenance likely occurs through the inhibition of cAMP signaling.**

Candida albicans, when a natural member of the commensal flora of healthy humans, occupies niches within mucosal membrane environments. *C. albicans* can also give rise to superficial mucosal infections such as oral and vaginal thrush, as well as life-threatening systemic infections in immunocompromised individuals (25). *C. albicans* is able to undergo morphological transitions between growth as yeast and filamentous forms (hyphae and pseudohyphae), and this morphological plasticity is influenced by numerous environmental signals (5). Morphogenesis is considered a key virulence trait of the fungus (36, 40), since strains locked in a given morphology exhibit attenuated virulence (40). Hyphal growth and the coordinated expression of hypha-specific genes are also important for virulence, as they promote attachment to biotic and abiotic surfaces (16, 35, 44), tissue invasion (17), and escape from phagocytic immune cells (28). Growth in the yeast morphology is thought to be important for dispersion *in vitro* (45) and may thus be significant during disseminated disease.

In both hypha-inducing liquid media and upon embedding in an agar matrix, Ras1-dependent activation of cyclic AMP (cAMP) production by adenylate cyclase (Cyr1) and subsequent activation of protein kinase A (PKA) are required for the induction of hyphal growth (14, 20). This induction likely occurs through activation of transcription factors by PKA (6) and decreased levels of the transcriptional repressor Nrg1, which acts in concert with Tup1 (8, 29). Ras1 is active in its GTP-bound state, and GTP binding is controlled by the guanine nucleotide exchange factor, Cdc25, which exchanges GDP for GTP; Ras1 GTPase activity is controlled by the GTPase activating protein, Ira2. As predicted, the *cdc25*-null mutant is afilamentous (13), whereas strains expressing a Ras1 variant (Ras1^{G13V}) that is locked in the GTP-bound conformation (15, 37) are hyperfilamentous. In addition, increases in cAMP signaling due to loss of the cAMP phosphodiesterase, Pde2, or increased Cyr1 activity result in hyperfilamentation (1, 3). In contrast, a mutant lacking the Cyr1-associated protein, Srv2 (formerly Cap1), with low intracellular cAMP levels is defective in embedded growth (2). During the first few minutes after transfer to hypha-inducing medium, a transient spike in intracellular cAMP is observed. Hyperfilamentation is also observed upon the loss of either Nrg1 or Tup1 (8), and recent data have shown that Nrg1 levels are negatively regulated by cAMP for several hours during early hyphal growth, but that they return to higher levels over time (29). Less is known about the roles of Ras1, cAMP signaling, and PKA activity in maintaining prolonged hyphal growth.

Farnesol, an extracellular quorum sensing molecule produced by *C. albicans* (22), represses the induction of hyphal growth by yeast cells in many different environments (11, 12, 23). This autoregulatory molecule prevents yeast cells from germinating through inhibition of the Ras1-cAMP pathway (11, 12) via direct inhibition of Cyr1 activity (19). While farnesol has been proposed to be a morphological regulatory signal, the effects of farnesol on the hypha-to-yeast transition are less clear. Ramage et al. (38) found that surface-associated *C. albicans* cells that had already begun to germinate or form hypha-containing biofilms were largely unaffected by the presence of high concentrations (300 μM) of farnesol, although farnesol inhibited biofilm formation when added along with yeast cells at the time of biofilm inoculation. Mosel et al. (32) likewise observed "farnesol resistance" when farnesol was added to germ tubes. Thus, a role for farnesol in affecting morphology in hyphae cells has not been demonstrated.

Here, our studies examined the effects of farnesol on *C. albicans* hyphae, and we used this molecule to determine whether the repression of cAMP signaling is sufficient to repress continued

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TABLE 1 Strains used in this study

Strain or plasmid	Genotype	Source or reference	Lab no.
Strains			
CAF2	Ura^+ derivative of CAI4	17	DH331
CDH107 (*ras1*Δ/Δ)	*ura3*Δ::λ*imm434*/*ura3*Δ::λ*imm434 ras1*::*hisG*/*ras1*::*hisG*::*URA3*	17	DH483
*ras1*Δ/Δ-*RAS1*	*ura3*Δ::λ*imm434*/*ura3*Δ::λ*imm434 ras1*::*hisG*/*ras1*::*hisG*::*RAS1-URA3*	37	DH1385
*ras1*Δ/Δ-$ras1^{G13V}$	*ura3*Δ::λ*imm434*/*ura3*Δ::λ*imm434 ras1*::*hisG*/*ras1*::*hisG*::$RAS1^{G13V}$-*URA3*	37	DH1658
BPS4 (*pde2*Δ/Δ)	*ura3*Δ::λ*imm434*/*ura3*Δ::λ*imm434 pde2*Δ::*hisG*/*pde2*Δ::*hisG eno1*::*URA3*/*ENO1*	2	DH128
BPS9 (*pde2*Δ/Δ-*PDE2*)	*ura3*Δ::λ*imm434*/*ura3*Δ::λ*imm434 PDE2*/*pde2*Δ::*hisG*/*pde2*Δ::*hisG eno1*::*URA3*/*ENO1*	2	DH1739
CR216 (*cyr1*Δ/Δ)	*ura3*Δ::λ*imm434*/*ura3*Δ::λ*imm434 cyr1*Δ::*hisG-URA3-hisG*/*cyr1*::*hisG*	39	DH346
*cyr1*Δ/Δ	*ura3*Δ::λ*imm434*/*ura3*Δ::λ*imm434 cyr1*Δ::*hisG*::*cyr1*Δ::*hisG*	This study	DH1666
*cyr1*Δ/Δ-*pTEF2*	*ura3*Δ::λ*imm434*/*ura3*Δ::λ*imm434 cyr1*Δ::*hisG*/*cyr1*Δ::*hisG-ura3*::pSM2/*URA3*	This study	DH1914
*cyr1*Δ/Δ-*pTEF2-CYR1*	*ura3*Δ::λ*imm434*/*ura3*Δ::λ*imm434 cyr1*Δ::*hisG*/*cyr1*Δ::*hisG-ura3*::pSMTC/*URA3*	This study	DH1916
BCa2-10 (*tup1*Δ/Δ)	*tup1*Δ::*hisG*/*tup1*Δ::p405-*URA3 ura3*Δ/*ura3*Δ	7	DH36
MMC3 (*nrg1*Δ/Δ)	*ura3*Δ::λ*imm434*/*ura3*Δ::λ*imm434 nrg1*::*hisG-URA3-hisG*/*nrg1*::*hisG*	33	DH49
Plasmids			
pSM2	*URA3* integrating plasmid	27	
pSMTC	pTEF2, full-length *CYR1* in pSM2	27	

hyphal growth. We found that farnesol promoted the hypha-to-yeast transition in both agar-embedded conditions and in liquid medium. However, mutants with increased signaling through the Ras1-Cyr1-PKA pathway or mutants defective for downstream repressors of hyphal growth were resistant to farnesol-enhanced hypha-to-yeast transition. In embedded conditions, the ability of farnesol to repress the maintenance of hyphal growth was enhanced at lower temperatures and decreased at higher temperatures. Although farnesol-mediated induction of the hypha-to-yeast transition appeared to be slower than farnesol-mediated inhibition of germination, these studies provide strong evidence that farnesol, through its effects on cAMP signaling, can promote the transition from hyphal growth to growth as yeast.

MATERIALS AND METHODS

Strains and growth conditions. The *C. albicans* strains used in the present study are listed in Table 1. Strains were streaked from freezer stocks stored at −80°C onto YPD (2% peptone, 1% yeast extract, 2% glucose) plates every 8 days. Overnight cultures were grown in 5 ml of YPD at 30°C in a roller drum for 16 to 18 h. Dimethyl sulfoxide (DMSO) was used to make 50 mM fresh stock solutions of *trans*,*trans*-farnesol or dodecanol (Sigma-Aldrich) before each experiment. The farnesol used to make the stock solutions was stored at 4°C in aliquots under nitrogen prior to use.

Strain construction. Strain CR216 (*cyr1*Δ/Δ) (39) was plated on 5-fluoroorotic acid to excise the *URA3* marker. The derivative of CR216 that was auxotrophic for uridine was complemented by electroporation-based transformation with HpaI-linearized pTEF2, a plasmid containing a full-length wild-type copy of the *CYR1* gene (18). For use as our empty vector control, pSM2 (18) was linearized with HpaI and then transformed by electroporation into the Uri^- derivative of CR216.

Embedded filamentation assay. Cells from overnight cultures were inoculated into fresh YPD to reach an optical density at 600 nm (OD_{600}) of 0.1 and then grown for 3 h at 30°C on a roller drum until the cultures reached an OD_{600} of ~0.3. The cells were then back diluted into YP. When farnesol was added at the time of inoculation, 25 ml of molten YPS agar (2% peptone, 1% yeast extract, 2% sucrose, 2% agar) was added to a 50-ml Falcon tube. A volume of YP containing 60 to 80 cells was then added to the tube, followed by 75 μM farnesol, 75 or 200 μM dodecanol, or an equal volume of DMSO (vehicle control) (11). The tube was then gently inverted to mix its contents and was then decanted into a 100-by-15-mm petri dish. The agar was allowed to solidify at room temperature, and the plates were incubated at 30°C.

When farnesol was added to 48-h-old hyphal colonies, the 15 ml of molten YPS agar containing *C. albicans* was poured into a petri dish, left to solidify at room temperature, and then incubated at 30°C for 48 h. Next, 10 ml of molten 2% agar (overlay) containing vehicle alone, 75 μM farnesol, or 200 μM farnesol was then added to the petri dish (see Fig. 3A), and then the plates were incubated at 23, 30, or 37°C for an additional 24 h.

Liquid assay. Stationary-phase cells from YPD overnight cultures were washed once with distilled water and then inoculated into 5 ml of YNBNP (0.67% yeast nitrogen base, 5 mM *N*-acetylglucosamine, 25 mM potassium phosphate buffer, 0.2% glucose); the cultures were then incubated at 37°C in a roller drum for 24 h. DMSO alone or DMSO with 75 μM farnesol was added to culture tubes within 3 h of germination.

Microscopy. Embedded colony morphologies were imaged after growth in agar for 24 to 72 h at ×10, ×20, ×60, or ×112.5 magnification with a Nikon SMZ1500 stereoscope. Representative colonies were imaged during the course of the experiment, and at least three biological replicates were completed for all experiments, obtaining reproducible results in each case. Higher-magnification images were also collected by excising a thin section from the colony periphery (still embedded in agar), which was then mounted onto a glass slide and subjected to DIC III imaging using a Zeiss Axiovert inverted microscope equipped with ×63 and ×100 objective lenses and Axiovision software. Similar sections were excised and boiled for 10 to 20 s in order to disperse cells for high-magnification imaging in order to ascertain the hypha-to-yeast/pseudohypha (PH) ratio at the colony periphery. For all experiments using sections excised from colonies, two sections were excised from three separate colonies, and 300 cells were counted per section. Analysis of the hypha-to-yeast/PH ratio was ultimately based on counts performed for three biological replicates. In these counts, each hypha (unicellular or multicellular) was counted as one unit. For liquid assays, 500 μl was transferred from the culture tube to a 1.5-ml tube, which was then vortexed in order to remove a representative sample for microscopic analysis using the inverted microscope.

RESULTS

***CYR1* is required for filamentation in embedded conditions, and farnesol, a Cyr1 inhibitor, blocks embedded hyphal growth.** Prior to using the embedded hyphal growth model to examine the effects of farnesol-mediated inhibition of Cyr1 in hyphae, we further characterized the embedded hyphal growth model with respect to the importance of Cyr1 signaling since there have been some differences among the *cyr1*Δ/Δ strain-embedded growth phenotypes reported (10, 14, 30). Cells within colonies formed by

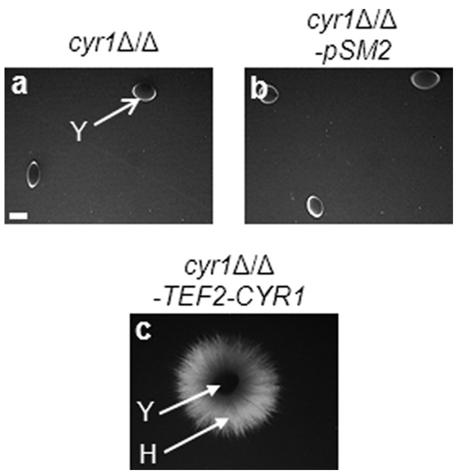

FIG 1 *CYR1* is required for the induction of filamentation in embedded growth conditions. The *cyr1*Δ/Δ mutant (a), the empty vector control (b), and the complemented strain (c) were grown in YPS agar at 30°C for 72 h and then imaged with a stereoscope at ×10 magnification. Scale bar, 1 mm. Y, yeast; H, hyphae.

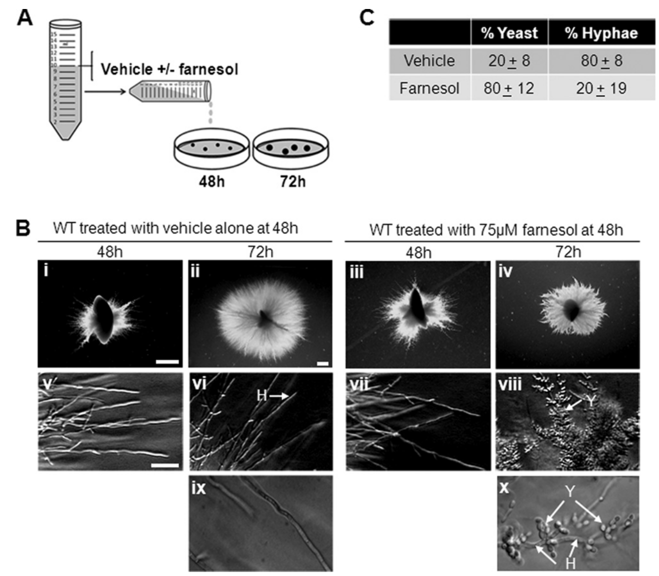

FIG 3 Farnesol enhances the hypha-to-yeast transition in embedded conditions. (A) Diagram depicting the overlay assay protocol used to treat preformed embedded colonies. (B) Wild-type colonies grown in YPS agar at 30°C for 48 h were imaged at ×20 magnification (i and iii). Immediately after imaging, an agar overlay was administered to deliver either vehicle alone (left panels) or vehicle with farnesol (right panels) to the preexisting colonies. At the 72 h time point (24 h after the overlay was applied), the colonies were imaged at ×10 magnification (ii and iv). The colony peripheries at 48 and 72 h were also imaged at ×112.5 magnification using a stereoscope (v to viii). For more detailed analysis of the periphery of 72-h-old colonies, images were also acquired using an inverted microscope equipped with a ×63 DIC III objective lens (ix and x). Scale bars, 0.5 mm. H, hyphae; Y, yeast. (C) For both treatment groups, two separate thin sections were isolated and, for each, 300 yeast and filaments in total were counted to determine the percent yeast and hyphae present. Hyphae (as opposed to hyphal cells) and yeast were counted for three colonies for each treatment group, and the average percentages of yeast and filaments are shown, with the standard deviations, for three biological replicates.

the *cyr1*Δ/Δ mutant remained exclusively in the yeast morphology over the course of 3 days (Fig. 1a and b), and restoration of the *CYR1* gene complemented the filamentation defect (Fig. 1c), allowing filamentation at levels comparable to the wild type (Fig. 2). Similarly, colonies formed by a strain lacking *RAS1* showed only very limited hypha formation after 3 days and this defect was complemented (see Fig. S1 in the supplemental material) (12, 15).

As previously shown, *C. albicans* wild-type cells that had been embedded in agar initially formed colonies comprised largely of yeast cells with sparse peripheral hyphae (Fig. 2a) (9). Over time, hyphae increasingly radiated from various regions of the colony (Fig. 2b and c). Consistent with the finding that Cyr1 was required for hyphal growth, the addition of farnesol, a Cyr1 inhibitor, to the agar at the time of inoculation prevented the appearance of hyphae at 24 h (Fig. 2d) and led to a marked reduction in the number and length of hyphae observed at 48 and 72 h (Fig. 2e and f). Hall et al. (19) have shown that farnesol blocks germination on solid media and in liquid cultures by inhibiting Cyr1 activity, while dodecanol exerts its effect on filamentation through induction of Sfl1, which is a negative regulator of hyphal growth. Our data suggest that dodecanol, which does not inhibit Cyr1 activity but potently inhibits hyphal growth in liquid through a Sfl1-dependent pathway (11, 21), had no significant effect on filamentation in embedded conditions when added at either 75 or 200 μM (see Fig. S2 in the supplemental material).

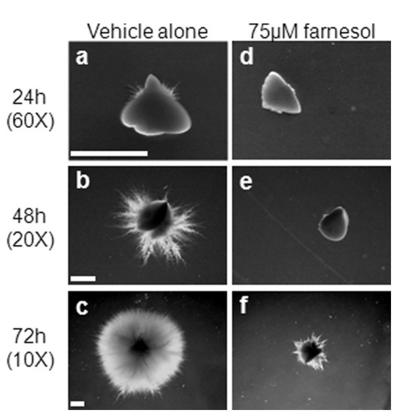

FIG 2 Farnesol inhibits hyphae emergence from embedded colonies. Wild-type colonies formed in YPS agar at 30°C with vehicle alone (a to c) or 75 μM farnesol (d to f) added at the time of inoculation. Colonies were imaged at ×60, ×20, and ×10 magnifications at 24, 48, and 72 h, respectively. Scale bars, 0.5 mm.

Farnesol enhances the hypha-to-yeast transition in preformed embedded colonies. Although farnesol clearly represses the induction of hyphal growth by yeast cells (11, 12, 19, 23, 37), its effects on hyphae had only been examined in detail over short time courses, and no effects were observed (32, 38). To determine the effect of farnesol on preexisting hyphae, we developed an assay in which an overlay containing either vehicle alone or farnesol was added to plates containing 48-h-old filamentous colonies (Fig. 3A). At 24 h after application of the overlay, hyphae at the periphery of farnesol-treated colonies were surrounded by abundant yeast, whereas hyphae predominated at the periphery of control colonies (Fig. 3B). The colonies in agar plates that received the overlay with vehicle were similar to those that did not receive additional top agar (Fig. 2c). Quantification of the cells in different morphologies in slices excised from the periphery revealed

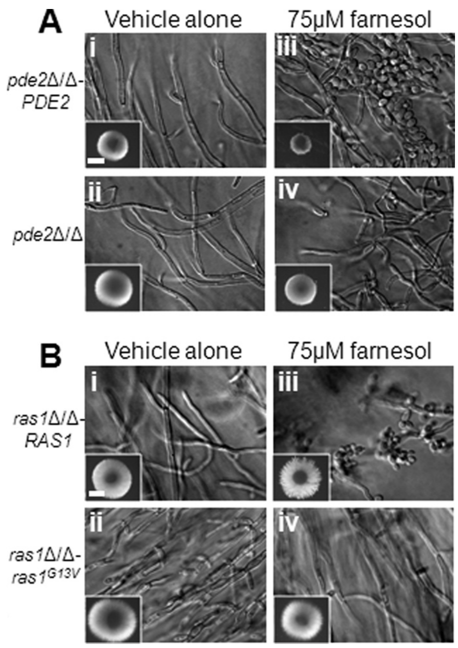

FIG 4 Artificially increased Ras1-cAMP signaling enhances resistance to farnesol in embedded conditions. (A and B) *pde2*Δ/Δ-*PDE2* and *pde2*Δ/Δ strains (A) and *ras1*Δ/Δ-*RAS1* and *ras1*Δ/Δ-*ras1*^{G13V} strains (B) grown in YPS agar at 30°C. Vehicle alone (i and ii) or farnesol (iii and iv) was added after 48 h of growth, and the colonies were imaged 24 h later. Whole colonies were imaged at ×10 magnification (insets). To evaluate the morphology at the colony periphery, images were also acquired using an inverted microscope equipped with a ×63 DIC III objective lens. Scale bar, 1 mm.

that colonies treated for 24 h with vehicle alone had 20% ± 8% yeast/PH ratios at the periphery, while those that received farnesol had 80% ± 12% yeast/PH ratios (Fig. 3C). These data indicate both that farnesol prevented robust increases in colony diameter and that this was in part due to enhanced lateral yeast growth from hyphae.

Artificial increases in Ras1-cAMP signaling enhances resistance to the effects of farnesol on the hypha-to-yeast transition in embedded conditions. To determine whether farnesol was increasing the hypha-to-yeast transition through the effects on cAMP signaling, the effects of farnesol on two strains with artificially increased levels of cAMP signaling were assessed. The first strain with increased cAMP signaling is the *pde2*Δ/Δ mutant, which has previously been described as being hyperfilamentous in embedded conditions (1). The *pde2*Δ/Δ mutant continued to filament even after application of the agar overlay with farnesol, whereas the *pde2*Δ/Δ-*PDE2* strain formed colonies with many yeast at the periphery (Fig. 4A). In colonies that received the vehicle alone, both the *pde2*Δ/Δ strain and the *pde2*Δ/Δ-*PDE2* strains had abundant hyphae (Fig. 4A). Quantification of the percentage of yeast versus PH and hyphae at the colony periphery in each strain revealed that the farnesol-treated *pde2*Δ/Δ mutant colonies contained 20% ± 7% yeast/PH, while the *pde2*Δ/Δ-*PDE2* strain contained 64% ± 10% yeast/PH, a difference confirmed to be statistically significant based on a Student *t* test ($P < 0.0001$). In the vehicle control cultures, the percentages of cells in different morphologies within colonies of the *pde2*Δ/Δ and *pde2*Δ/Δ-*PDE2* strains were not significantly different (2% ± 4 and 11% ± 6% yeast/PH, respectively). In addition to the 3-fold-greater abundance of lateral yeast/PH, the *pde2*Δ/Δ-*PDE2* colonies showed less expansion in the presence of farnesol compared to the *pde2*Δ/Δ strain (Fig. 4A, insets).

The second strain with increased cAMP signaling bore the *ras1*^{G13V} allele that encodes a Ras1 variant that is stabilized in the active Ras1 GTP-bound conformation (37) which hyperactivates the cAMP-PKA pathway. The *ras1*Δ/Δ mutant complemented with *RAS1* formed filamentous colonies (Fig. 4B) similar to those formed by the wild type (Fig. 3B), and colonies contained 62% ± 9% yeast/PH in the presence of farnesol (Fig. 4B). In contrast, the hyperfilamentous *ras1*Δ/Δ-*ras1*^{G13V} strain continued to form filaments in the presence of farnesol (Fig. 4B) with 29% ± 6% yeast/PH, and this difference was significant ($P < 0.0009$). Because higher concentrations of farnesol (200 μM) have been used previously, we also tested this concentration for its effects on yeast formation from hyphae in the *pde2*Δ/Δ and *ras1*Δ/Δ-*ras1*^{G13V} strains. In neither case did incubation in this high concentration of farnesol promote further production of lateral yeast at the periphery of colonies (data not shown).

Studies conducted in liquid growth conditions have shown that the cAMP pathway transiently represses levels of Nrg1 (29), a DNA-binding protein that interacts with the transcriptional factor Tup1 to repress transcription of hypha-specific genes (8). Both *tup1*Δ/Δ and *nrg1*Δ/Δ mutants are resistant to farnesol treatment when grown on top of agar at 30°C, and they secrete high levels of farnesol at 37°C (24). Our studies revealed that neither the *tup1*Δ/Δ strain nor the *nrg1*Δ/Δ strain formed lateral yeast in a manner similar to the wild type under embedded conditions in the presence of exogenous farnesol (see Fig. S3 in the supplemental material). However, microscopic analysis of cells at the colony periphery found an increased percentage of cells in the pseudohyphal morphology (see Fig. S3 in the supplemental material).

Low temperatures enhance the hypha-to-yeast transition in embedded conditions. It has been well established that hyphal growth in liquid conditions is strongly influenced by temperature, with the formation of true hyphae being highly stimulated at 37°C. Through Hsp90, temperature has been shown to impact hyphal growth controlled by the Ras1-Cyr1-PKA cascade (41). Although body temperature is not a requirement for hyphal growth in embedded conditions, we found a positive correlation between temperature and the maintenance of filamentation in embedded colonies in the presence of farnesol. Here, we allowed colonies to grow for 48 h at 30°C, applied an agar overlay containing vehicle alone or farnesol, and then incubated the colonies for an additional 24 h at 23, 30, or 37°C. Temperature alone had a modest effect on morphology, with more yeast cells at 23°C compared to 30 or 37°C (Fig. 5i to iii). Farnesol, when added to filamentous colonies, greatly exaggerated the effects of temperature. At 23°C, farnesol induced the formation of huge bunches of lateral yeasts (Fig. 5iv), whereas farnesol had a minor effect on cellular morphology at 37°C. Quantitation of cells at the colony peripheries indicated that farnesol treatment in combination with incubation at 23, 30, or 37°C resulted in 83% ± 6%, 67% ± 18.8%, or 50% ± 3.9% of cells in the yeast/PH morphology, respectively. This indicated that the formation of lateral yeast decreased as temperature increased (Fig. 5vi). In addition, we found that farnesol impacted colony expansion more drastically in colonies incubated at lower temperatures (Fig. 5, insets). These findings suggest that specific cues, such as temperature, modulate cAMP signaling and perhaps the response to farnesol.

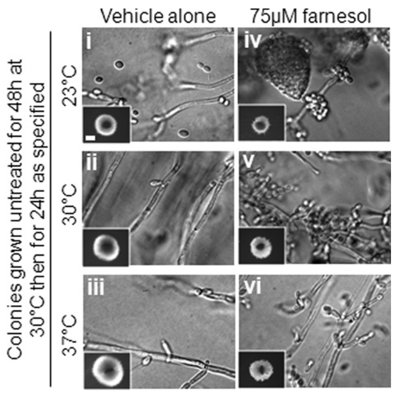

FIG 5 The effect of farnesol on the hypha-to-yeast transition varies inversely with temperature. Wild-type colonies were grown in YPS-agar at 30°C for 48 h, and then an agar overlay was administered to deliver either vehicle alone (i to iii) or farnesol (iv to vi) to the preexisting colonies. The plates were then incubated for an additional 24 h at 23°C (i and iv), 30°C (ii and v), or 37°C (iii and vi). At the 72-h time point (24 h after the overlay was applied), a stereoscope was used to image colonies at ×10 magnification (insets), and the periphery was then examined for the hypha-to-yeast transition using an inverted microscope equipped with a ×63 DIC III objective lens.

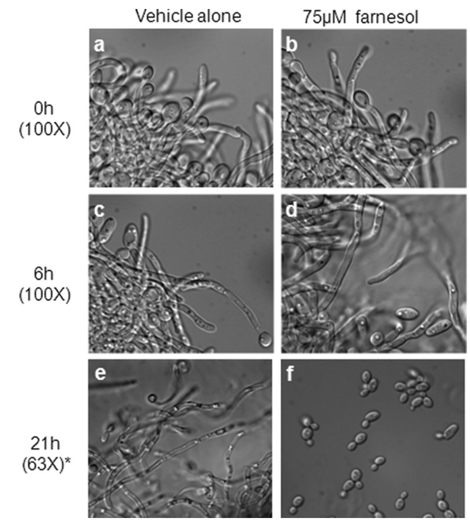

FIG 6 Effect of farnesol on hypha elongation in liquid-inducing conditions. Wild-type (SC5314) germination was induced by growth for 3 h in YNBNP in a roller drum at 37°C. (a and b) Once germ tubes had formed, designated "0 h," the cultures were imaged. Germ tubes were then treated with 75 μM farnesol or vehicle alone and returned to the 37°C roller drum. Images were subsequently collected at 9 h (c and d) and 21 h (e and f) using either at ×100 (a to e) and ×63 (f and g) objective magnifications. *, The magnification was decreased to ×63 in order to increase the field of view to allow the visualization of more cells.

Farnesol induces the hypha-to-yeast transition in wild-type *C. albicans* in liquid medium. The studies described above using the embedded assay showed that farnesol induces the formation of yeast from hyphae and that factors that perturb Ras1-Cyr1 signaling modulate the strength of the farnesol response. To determine whether farnesol induces the hypha-to-yeast transition in liquid medium, the SC5314 wild-type strain was grown in hypha-inducing medium for 3 h and then challenged with either farnesol or vehicle alone. For cultures that received vehicle treatment only, large hyphal aggregates dominated the cultures (Fig. 6). In contrast, farnesol led to the formation of shorter hyphae, some pseudohyphae, and a significant population of yeast within 6 h of treatment (Fig. 6d), with yeast predominating 21 h posttreatment (Fig. 6f). Macroscopic analysis of these cultures showed that, while control cultures contained large aggregates that quickly settled to the bottom of the tubes leaving a relatively clear supernatant, the farnesol treated cultures maintained a semiturbid milky appearance (data not shown). These data suggest that farnesol induces the hypha-to-yeast transition in liquid over time. As in embedded conditions, the *pde2*Δ/Δ and *ras1*Δ/Δ-*ras1*^{G13V} strains, which have increased cAMP signaling, were more resistant to the effects of farnesol in liquid growth conditions compared to their reference strains (Fig. 7).

DISCUSSION

Because the ability of *C. albicans* to switch between growth in yeast and filamentous forms is crucial to virulence (28, 36, 40), the pathways that control the yeast-to-hypha transition have been intensely examined. Previous work has shown that the Ras1-Cyr1-PKA pathway is important in stimulating the yeast-to-hypha transition in response to various environmental cues (15, 27, 39, 43), and that the autoregulatory molecule farnesol represses the induction of hyphal growth by inhibiting Cyr1 (19). Cyr1 activity is required for the generation of a spike in cAMP levels (14, 30) that activates Tpk1 and Tpk2, which are required for hyphal growth (6, 32, 43). Activation of the cAMP pathway also results in a transient decrease in Nrg1, a negative transcriptional regulator of hyphal growth (29). Neither high cAMP levels nor low Nrg1 levels are maintained throughout hyphal growth.

The role of cAMP signaling or farnesol in the maintenance of hyphal growth or the hypha-to-yeast transition is much less clear. Here, we present several pieces of evidence that suggest the Ras1-Cyr1-PKA pathway is also involved in maintaining hyphal growth

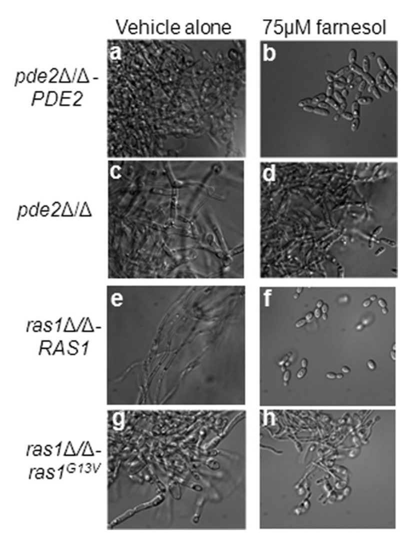

FIG 7 Artificially increased Ras1-cAMP signaling enhances resistance to the hypha-to-yeast transition in liquid conditions. Germination of the *pde2*Δ/Δ-*PDE2* (a and b), *pde2*Δ/Δ (c and d), *ras1*Δ/Δ-*RAS1* (e and f), and *ras1*Δ/Δ-*ras1*^{G13V} (g and h) mutants was induced by growth for 3 h in YNBNP in a roller drum at 37°C. After germ tube formation (0 h), the cultures were treated with vehicle alone or 75 μM farnesol and returned to the 37°C in a roller drum. Images were subsequently collected at 21 h using an inverted microscope equipped with a ×63 DIC III objective lens.

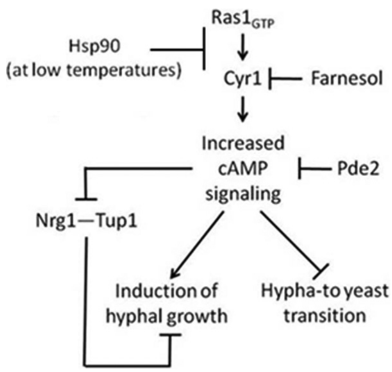

FIG 8 Cyr1 activity inhibits the hypha-to-yeast transition in both embedded and liquid growth conditions. The proposed model indicates Cyr1 activity is required to maintain hyphal growth. The hypha-to-yeast transition results from downregulation of cAMP signaling due to inhibition of Cyr1 activity by (i) Hsp90 activity at low temperatures and (ii) exogenously added farnesol.

and that farnesol induces the hypha-to-yeast transition by inhibiting this pathway. First, the addition of farnesol to preexisting filamentous colonies embedded in agar resulted in a striking increase in lateral yeast formation and decreased colony expansion (Fig. 3). Second, while previous studies revealed no effect of farnesol on germ tube extension formation during a 3.5-h experiment in liquid medium (32), we detected yeast cells forming from hyphae 6 h posttreatment (Fig. 6 and 7). Third, mutants with increased cAMP signaling, due to deletion of the *PDE2* genes or the presence of constitutively active Ras1G13V, were more resistant to farnesol-mediated induction of hypha-to-yeast transitions in both solid (Fig. 4) and liquid (Fig. 7) media, and this is consistent with previously published observations that these strains are hyperfilamentous (1, 15, 37). Lastly, *tup1* and *nrg1* mutants lacking negative regulators of hyphal growth that are controlled, at least in part, by elements downstream of the Ras1-cAMP signaling (29) continued to filament in the presence of farnesol (see Fig. S3 in the supplemental material). These results are summarized in the model proposed in Fig. 8. Together, these data suggest that Cyr1 activity is required to maintain filamentation in liquid and embedded conditions and that its activity in hyphae can be inhibited by farnesol, although more time is required for farnesol to exert an effect on morphology once filamentation has begun.

Our finding that temperature changes the sensitivity of *C. albicans* to farnesol (Fig. 5) is in agreement with previous reports that temperature influences Hsp90, a molecular chaperone that inhibits the function of the Ras1-Cyr1-PKA pathway (41). Interestingly, farnesol was able to enhance the hypha-to-yeast transition by filaments formed in liquid medium at 37°C (Fig. 6 and 7), although this effect was less striking at the same temperature in embedded conditions (Fig. 5). These observations parallel previous work by Langford et al. (26), who demonstrated that culture conditions and growth stage influence farnesol-mediated inhibition of growth. The temperature-dependent responses of cells to farnesol may be due to differences in the activity levels of the Ras1-cAMP-PKA pathway and whether inhibition is sufficient to cause phenotypic changes.

Repression of the hyphal growth program appears to be a two-part process that involves (i) inhibition of filament elongation and (ii) the production of lateral yeast cells from preexisting hyphae. Although farnesol treatment did not appear to enhance the hypha-to-yeast transition in the *pde2*-, *tup1*-, and *nrg1*-defective strains, it did result in a significant decrease in colony expansion. This may be indicative of farnesol effects on hyphal cell elongation. Because strains expressing Ras1G13V still formed large colonies in the presence of farnesol (Fig. 4B), Ras1-controlled pathways may promote hyphal extension. Future studies will determine how farnesol-mediated inhibition of cAMP signaling may promote the hypha-to-yeast transition and inhibit hyphal cell elongation through other regulators such as Eed1, the epithelial escape and dissemination gene, and Ume6, which are required to maintain hyphal growth (4, 31, 46), or the *C. albicans pescadillo* homolog Pes1 shown by Shen et al. (42) to be required for the formation of lateral yeast growth from hyphae.

Farnesol production by *C. albicans* correlates with increased virulence in a systemic mouse model of candidiasis (34). Future work will determine how the physical and chemical environments in host fluids and tissues influence cAMP signaling, *C. albicans* farnesol production, and the ability to respond to farnesol and whether these responses are relevant to invasion and dispersal (45) in the context of disease.

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